Journal of Surgical Oncology 62:279–283 (1996)

p53 Protein Expression in Pancreatic Tumors and Its Relationship to Clinicopathological Factors and Prognosis

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We examined the expression of p53 protein by immunohistochemical method in a series of pancreatic tumors and evaluated its relationships to the clinicopathological factors and prognosis. The study involved 108 cases of pancreatic tumors (79 ductal carcinomas, 1 acinar cell carcinoma, 14 endocrine tumors, 6 solid cystic tumors, 8 benign ductal tumors) and 8 chronic pancreatitides. Thirty-nine cases of pancreatic ductal carcinoma (49.4%) were positive for p53 protein. Analysis of the Cox hazards model identified p53 positivity and stage at the initial operation as an independent prognostic factor. Patients with p53 positive ductal carcinomas had a greater risk of death compared to p53 negative cases ($P < 0.05$). There was, however, no statistically significant correlation between p53 protein expression and other clinicopathological factors. Cases of stage III and IVb with positive p53 showed a bleak prognosis compared to p53 negative cases ($P < 0.05$). Our results suggest that p53 expression is common in invasive pancreatic ductal carcinomas and may have a prognostic value.

KEY WORDS: **tumor suppressor oncogene, pancreatic carcinoma, immunohistochemistry, clinical behavior**

INTRODUCTION

The human p53 gene is located on the short arm of chromosome 17 (17p13) and consists of 20 kb genomic DNA with 11 exons [1]. The p53 gene product was first identified as a host protein to bind with simian virus 40 (SV40) large T antigen (2). It encodes a 53-kD nuclear phosphoprotein with cell cycle-regulatory and transcriptional function [1,3]. Since wild-type p53 gene products reduce transforming activity of many oncogenes to suppress tumor growth [4], p53 is widely known as a tumor suppressor gene in its wild-type form. In contrast to a short half time (20–30 min) of wild-type p53 protein, mutant p53 is more stable and has a long half time (4–8 hr) [1]. Such a biological character enables us to detect mainly the mutant p53 protein on histological sections by immunohistochemistry [5]. The mutation and inactivation of p53 gene have been demonstrated at high frequency in various human tumors, including colon [6,7], stomach [8,9], lung [10], urinary bladder [11], and breast cancers [12].

Recent studies also have disclosed an abnormal expression of p53 suppressor gene in 40–60% cases of pancreatic adenocarcinoma [13–18]. These studies addressed only the pancreatic duct cell tumors, and the significance of p53 alterations has yet to be determined. We have, therefore, examined p53 protein expression in benign and malignant pancreatic tumors by immunohistochemical method and explored its relationship to the clinicopathological features and prognosis.

MATERIALS AND METHODS

The study involved 108 cases of pancreatic tumors and 8 chronic pancreatitides (Table I). All specimens for the histological studies were obtained at operation or laparo-

Accepted for publication March 6, 1996.
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TABLE I. p53 Protein Expression in Pancreatic Tumor

	Total	p53 positive (%)	Grade of positivity[a]				
			1+	2+	3+	4+	(<5%)
Ductal carcinoma	79	39 (36.1)	13	8	9	9	(1)
Acinar cell tumor	1	0	0	0	0	0	(0)
Endocrine cell tumor	14	0	0	0	0	0	(4)
Solid cystic tumor	6	0	0	0	0	0	(0)
Intraductal papillary adenoma	1	0	0	0	0	0	(0)
Serous cystadenoma	2	0	0	0	0	0	(0)
Mucinous cystadenoma	5	0	0	0	0	0	(0)
Chronic pancreatitis	8	0	0	0	0	0	(0)
Total	116	39 (33.6)	13	8	9	9	(5)

[a]Positivity of the grade is based on the percentage of stained nuclei (see text).

tomic biopsy and processed for formalin-fixation and paraffin-embedding. Sections were cut at 4-μm thickness and stained with hematoxylin and eosin. Serial sections were used for immunohistochemical stains with a monoclonal antibody against p53 (DO-1, Oncogene Science, Uniondale, NY). The classification and histological differentiation were based on the criteria according to the general rules for the study of pancreatic cancer, reported by the Japan Pancreas Society [19].

For the detection of p53, heating treatment with microwave oven (600 W, 2 min × 5 times in 0.01 M citrate buffer pH 6.0) was employed for the special retrieval of antigen after deparaffinization [20]. Endogenous peroxidase activity was blocked by incubation in 0.3% H_2O_2 in methanol for 30 min. The sections were then pretreated with normal rabbit serum for 30 min to reduce nonspecific staining. Thereafter, the sections were incubated with a primary antibody of 1:200 dilution at 4°C overnight. Following the incubation with biotinylated antimouse immunoglobulins at room temperature for 30 min, they were reacted with streptavidin-biotin-peroxidase complex (Histofine SAB-PO, Nichirei Co., Tokyo) at room temperature for 30 min. Reaction products were visualized with 3,3′-diaminobenzidine tetrahydrochloride. Nuclei were counterstained with 1% methylgreen solution buffered with veronal acetate.

When the percentage of cells with clear nuclear staining exceeded 5% of 1,000 neoplastic cells, the case was considered positive for p53 expression, as similarly determined in a previous report [21], although others defined positive as the case showing only a few cells [8,9] or 20% [20]. Positive p53 staining was graded by the percentage of positive cells per 1,000 neoplastic cells as 1+(<5–25%), 2+(25–50%), 3+(50–75%), and 4+(75%<).

The relationships of p53 protein expression were evaluated with respect to clinical data, histologic type, degree of histological differentiation, and stage of the tumors by Mann-Whitney's U test or X^2 test.

Survival data was available in 31 cases, in which follow-up time ranged up to 64 months. The Cox proportional hazards model was used to identify which independent factors had a significant influence on patient survival. Analysis of survival was also performed using the Kaplan-Meier method, in which the generalized Wilcoxon test was used for statistical comparisons. Statistical significance was obtained when the P value was <0.05.

RESULTS

Thirty-nine of 79 cases (49.4%) of pancreatic duct cell carcinomas were positive for p53 protein (Table I). The distribution of stained nuclei was varied in cases from focal to diffuse (5–92.8%) (Table I, Fig. 1a,b). Four of 14 cases 28.6% of pancreatic endocrine tumors, although designated as negative, showed focal immunoreactivities for p53 protein in a small percentage of cells (0.3–1.7%) (Table I, Fig 1c). p53 reactions were negative in acinar cell carcinoma, intraductal papillary adenomas, serous, or mucinous cystadenomas and solid cystic tumors. No positive staining was detected in eight cases of chronic pancreatitis (Table I).

p53 protein expression was detected in all histologic types of ductal cell carcinoma; 2 of 6 cases (33.3%) in papillary adenocarcinomas, 24 of 46 (52.2%) in tubular adenocarcinomas, 1 of 3 in adenosquamous carcinomas, 2 of 6 in anaplastic carcinomas, 4 of 8 in intraductal papillary adenocarcinomas, and 6 of 10 in mucinous cystadenocarcinomas (Table II). Cases with obvious invasion showed positive immunoreactivities in 4 of 4 cases of intraductal papillary adenocarcinomas and 5 of 8 cases in mucinous cystadenocarcinomas.

With respect to the histological differentiation, there was a trend toward a higher positivity in poorly differentiated type as compared to well and moderately differentiated types. The grade of positively stained nuclei also tended to be increased in the poorly differentiated type (Table III), although no statistical significance was obtained among the three groups. There were no statistically significant associations between p53 protein expression and clinicopathological factors, such as lymphatic inva-

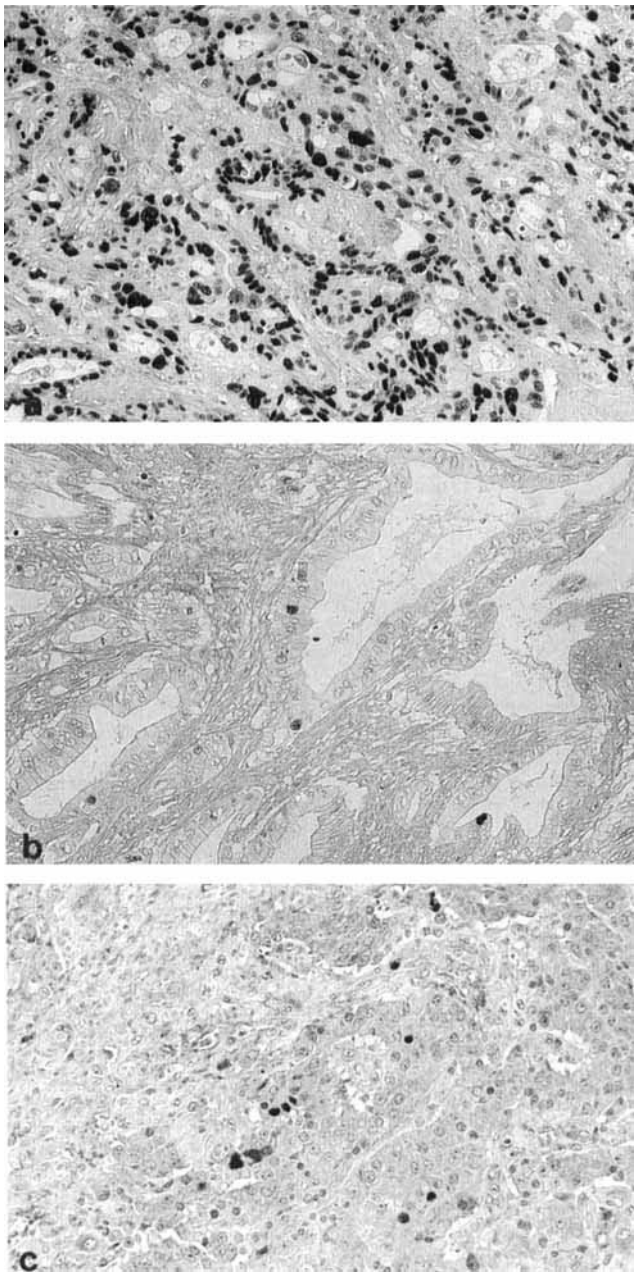

Fig. 1. Immunohistochemical staining of p53 in pancreatic ductal carcinoma **(a,b)** and endocrine cell tumor **(c)**. (a) Poorly differentiated tubular adenocarcinoma; most of the tumor cell nuclei are positive for p53 protein (4+, 80.5% of cells are positive). (b) Well-differentiated tubular adenocarcinoma; focal positive staining of p53 protein are seen (1+, 10.3% of cells are positive). (c) Endocrine cell tumor; there are a few positive cells but it was designated as negative since <5% (1.5%) of cells are positive.

sion, venous invasion, perineurial invasion, lymph node metastasis, tumor size as well as the stage.

The Cox hazards model using 8 variables as shown in Table IV revealed that p53 protein expression and stage at the initial operation were significant unfavorable prognostic factors (Table IV). Age, sex, histological differentiation, tumor size, lymphnode metastasis, and type of surgery did not have independent prognostic value. The median survival was significantly shorter in patients with p53 positive carcinoma (12.1 months) than in p53 negative cases (34.1 months) ($P = 0.019$) (Fig. 2a). In patients with pancreatic carcinoma of stages III and IVb, there was a statistical significance in the median survival between p53 positive and negative cases ($P = 0.026$, $P = 0.033$) (Fig. 2b,c), whereas at stage IVa, the difference did not reach statistical significance ($P = 0.059$).

DISCUSSION

In the present study, 49.4% of duct cell carcinomas were positive for p53 protein. This percentage was comparable to the previous data showing 40–60% positivity in pancreatic cancers [13–18]. It has been suggested that mutation of p53 gene may be a relatively early event in the pancreatic tumorigenesis [16,17]. In our study, however, we found positive p53 protein expression in minimally invasive ductal carcinomas as well as invasive carcinomas, but not in noninvasive ductal carcinoma and adenoma. It may be speculated that our immunohistochemical staining was not sensitive enough to detect small expression of mutated p53 antigen in noninvasive carcinoma. Alternatively, p53 expression detectable at the routine immunohistochemistry may be more relevant to the invasive behavior of pancreatic carcinoma rather than the early oncogenesis. Although the data of pancreatic tumors cannot simply be applied to other sites, studies on breast carcinomas [12] as well as colorectal adenocarcinomas [22] have shown a higher positivity of immunoreactive p53 expression in advanced stages than in the less advanced stage. Since our cases are too few to draw a conclusion for the significance of p53 expression, further studies are needed.

In the present study, the Cox hazards model showed that p53 expression and stage at the initial operation had independent prognostic values. By Kaplan-Meier analysis, the median survival in cases of stage III and IVb was shorter when p53 was positive compared to p53 negative cases. This trend was also found in cases of stage IVa. Previous studies could not find a significant correlation between p53 protein expression and prognosis in pancreatic cancers [15,16]. This is probably due to the small number of patients or lack of consideration of the stage for the statistical analysis in previous studies. Mutation of p53 may inhibit regulation of celluar growth by apoptosis and thereby promote the progressive proliferation of neoplastic cells in pancreatic carcinomas [23]. Such cellular events may eventually associate with poor prognosis of the patients.

In this study we used formalin-fixed and paraffin-embedded tissues for immunohistochemistry. These samples are not always appropriate for the preservation of antigenicity. Microwave oven heating is a useful pretreatment

TABLE II. p53 Expression of Pancreatic Ductal Carcinoma

Histological classification[a]	No. of tumors	p53 positive (%)
Papillary adenocarcinoma	6	2 (33.3)
Tubular adenocarcinoma	46	24 (52.2)
Adenosquamous carcinoma	3	1 (33.3)
Anaplastic carcinoma	6	2 (33.3)
Intraductal papillary adenocarcinoma	8	4 (50.0)
non-invasive	1	0
minimally invasive	3	0
invasive	4	4 (100)
Mucinous cystadenocarcinoma	10	6 (60.0)
non-invasive	1	0
minimally invasive	1	1 (100)
invasive	8	5 (62.5)
Total	79	39 (49.4)

[a]According to the classification of the general rules for the study of pancreatic cancer of the Japan Pancreas Society.

TABLE III. p53 Expression and Histological Differentiation of Pancreatic Ductal Carcinoma

			Grade of positivity[a]				
	No. of tumors	p53 positive (%)	1+	2+	3+	4+	(<5%)
Well differentiated	15	6 (40.0)	2	1	2	1	(1)
Moderately differentiated	30	18 (60.0)	5	6	3	4	(0)
Poorly differentiated	11	8 (72.7)	2	0	3	3	(0)
Total	52	30	9	7	8	8	(1)

[a]Positivity of the grade is based on the percentage of the number of stained nuclei.

TABLE IV. Cox Proportional Hazards Model for Factors Associated With Survival of Patients With Pancreatic Ductal Carcinoma

Variables		Hazard ratio	*P* value
p53 immunostaining	(positive/negative)[a]	23.101	0.0003
Age (years)	($60 \leq y/\geq 60$ y)	2.144	0.2421
Sex	(male/female)	0.465	0.2869
Histological differentiation	(pap,well/mod,por)[b]	1.227	0.7625
Tumor size	(TS1,2/TS3,4)	1.029	0.9763
Stage	(stage I,II,III/stage IVa,b)[c]	6.946	0.0268
Lymph node metastasis	(n+/n−)	4.605	0.0700
Type of surgery	(operation/biopsy)	0.167	0.1127

[a]Positivity of the grade is based on the percentage of stained nuclei.
[b]Histological differentiation is classified into a differentiated group consisting of papillary (pap) and well-differentiated (well) carcinoma and a less differentiated group consisting of moderately differentiated (mod) and poorly differentiated (por) carcinoma.
[c]Tumor size and stage are graded according to the classification of the general rules for the study of pancreatic cancer by the Japan Pancreas Society.

for the retrieval of antigens in immunohistochemical studies [20]. We confirmed the value of this method for the detection of p53 protein, because the positive reaction was enhanced 50% as compared to the tissues without pretreatment (Aizawa, unpub. obs.).

Four of 14 cases (28.6%) of pancreatic endocrine tumors showed weak expression of p53 protein, although the reaction was all focal and determined as negative (0.3–1.7%). In previous studies, p53 mutations were not detected in pancreatic endocrine tumors and acinar cell carcinomas [24,25]. It may be possible that weak expression of p53 protein in endocrine tumors, as shown in this study, may be a wild-type rather than a mutated protein, because p53 protein may often accumulate without muta-

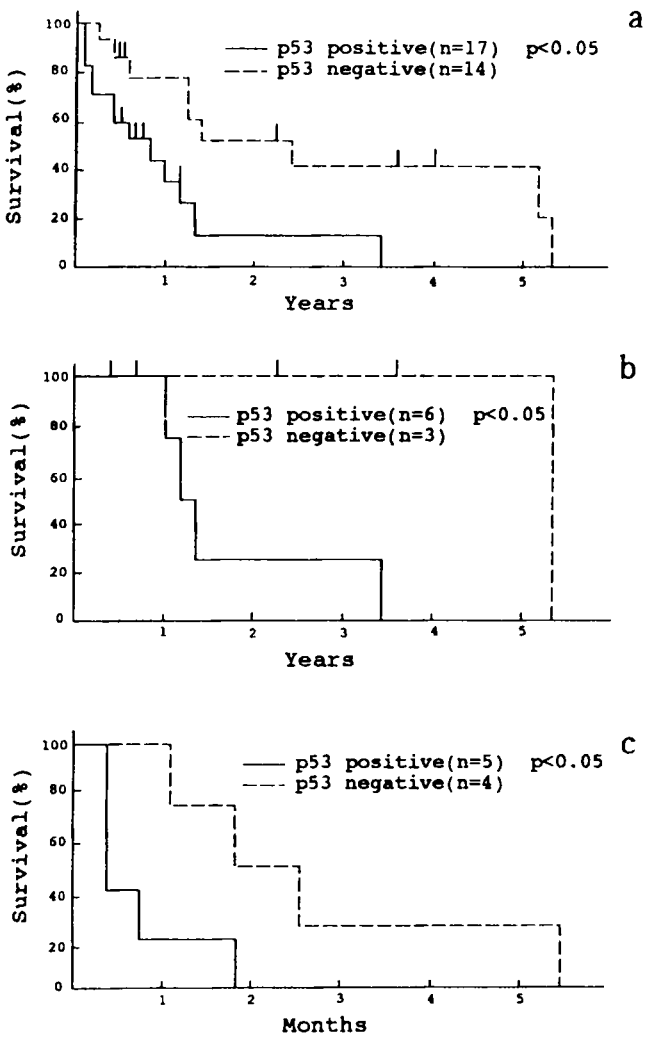

Fig. 2. Survival analysis of patients in relation to p53 expression. p53 positive cases show a significantly shorter duration than p53 negative cases **(a)**. Cases of stage III **(b)** and IVb **(c)** also show a shorter survival when they are positive to p53 as compared to p53 negative cases.

tion, as a protective response to DNA damage (G1 arrest) [3] or as a result of stabilization of normal p53 protein by complex formation with a cellular oncoprotein such as mdm2 [26]. When the percentage of immunoreactive cells is low, it is likely that focal positivity reflects wild-type p53 protein. Further investigation of gene analysis of p53 mutation is needed in such cases to confirm that such hypothesis is true.

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